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# BDNF Val66Met and DRD2 Taq1A polymorphisms interact to influence PTSD symptom severity: A preliminary investigation in a South African population

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## Abstract

*Background:* We evaluated the role that selected variants in serotonin transporter (*5-HTT*), dopamine receptor 2 (*DRD2*) and brain-derived neurotrophic factor (*BDNF*) genes play in PTSD symptom severity in an at-risk population. We also investigated the interaction between the genetic variants to determine whether these variables and the interactions between the variables influenced the severity of PTSD symptoms.

*Methods:* PTSD symptoms were quantitatively assessed using the Davidson Trauma Scale (DTS) in 150 participants from an at-risk South African population. All participants were genotyped for the *5-HTTLPR*, *DRD2 Taq1A* and *BDNF Val66Met* polymorphisms. Gene–gene interactions were investigated using various linear models. All analyses were adjusted for age, gender, major depressive disorder diagnosis, level of resilience, level of social support and alcohol dependence.

*Results:* A significant interaction effect between *DRD2 Taq1A* and *BDNF Val66Met* variants on DTS score was observed. On the background of the *BDNF Val66Val* genotype, DTS score increased significantly with the addition of a *DRD2 Taq1A A1* allele. However, on the *BDNF Met66* allele background, the addition of an *A1* allele was found to reduce total DTS score.

*Conclusions:* This study provides preliminary evidence for an epistatic interaction between *BDNF Val66Met* and *DRD2 Taq1A* polymorphisms on the severity of PTSD symptoms, where both too little and too much dopamine can result in increased PTSD symptom severity.

## 1. Introduction

Posttraumatic stress disorder (PTSD) is an anxiety disorder that develops following exposure to a life-threatening event (APA, 1994). However, not all trauma-exposed individuals develop PTSD. The lifetime prevalence of exposure to traumatic events varies between 40% and 90%, depending on the sample investigated, whereas the lifetime prevalence of PTSD in trauma-exposed individuals has been estimated at approximately 9% (Breslau et al., 2012). Numerous risk factors for developing PTSD have been elucidated, including those pertaining to the traumatic event, such as type, severity and duration of trauma, poor social support and childhood adversity (reviewed in Yehuda and LeDoux, 2007). In addition,

twin and family studies have indicated that genetics plays an important role in the development of PTSD, with heritability estimates between 30% and 40% (Sack et al., 1995; Stein et al., 2002; True et al., 1993; Yehuda et al., 1998, 2001, 2002). Although a number of candidate gene association studies have been conducted to identify susceptibility variants playing a role in the aetiology of PTSD (reviewed in Cornelis et al., 2010), no gene variant has yet been reported as being unequivocally involved in the development of the disorder. One of the reasons for the inconclusive results may be that few studies have accounted for environmental factors that potentially influence the development of PTSD. In addition, to date, no studies have investigated the potential effects that interactions between genetic variants may have on influencing susceptibility to PTSD.

The most widely studied polymorphism in PTSD genetics is one that occurs in the promoter region of the gene encoding the serotonin transporter (*5-HTT*), which plays a crucial role in regulating serotonergic activity in the synapse. The *5-HTT*-linked polymorphic region (*5-HTTLPR*) is a 44 base pair (bp) insertion/deletion polymorphism characterised by either 14 (short, *S*) or 16 (long, *L*) copies of an imperfect 22–23 bp repeat (Heils et al., 1996; Lesch et al., 1996). The *S* allele has been found to possess transcriptional activity two to three fold lower than that of the *L* allele (Lesch et al., 1996). Of the ten candidate gene association studies that have investigated the role that *5-HTTLPR* plays in the development of PTSD, five have yielded significant associations between the lower-expressing allele (*S* allele) and PTSD (Kilpatrick et al., 2007; Koenen et al., 2009; Kolassa et al., 2010; Lee et al., 2005; Xie et al., 2009). However, three studies yielded results implicating the higher-expressing allele (*L* allele) in the pathogenesis of PTSD (Grabe et al., 2009; Sayin et al., 2010; Thakur et al., 2009), while two studies did not find any association between *5-HTTLPR* and PTSD (Mellman et al., 2009; Valente et al., 2011).

A number of lines of evidence implicating dopamine in the development of PTSD also exist. Dopamine has been detected at elevated amounts in urine and plasma samples from PTSD patients compared to controls (Hamner and Diamond, 1993; Lemieux and Coe, 1995; Yehuda et al., 1992). The dopamine receptor 2 gene (*DRD2*) possesses a potentially functional single nucleotide polymorphism (SNP), *DRD2 Taq1A* (rs1800497), in the 3′UTR (Grandy et al., 1989). The SNP comprises two alleles, namely a *T* allele, known historically as the *A1* allele, and a *C* allele, known historically as the *A2* allele. For the sake of parity with previous publications, the current study will make use of the *A1*/*A2* allelic nomenclature. Numerous studies have indicated that *A1* allele carriers exhibit a reduced number of DRD2 in the brain (Jonsson et al., 1999; Noble et al., 1991; Pohjalainen et al., 1998; Ritchie and Noble, 2003; Thompson et al., 1997) and increased rates of dopamine synthesis rates in the striatum (Laakso et al., 2005). The *DRD2 Taq1A* variant has been investigated for its role in the development of PTSD in a number of studies, with inconsistent results. A significant association has been observed between the *A1* allele and PTSD in a cohort of European American Vietnam war veterans (Comings et al., 1996), although these results were not replicated in a subsequent study (Gelernter et al., 1999). On the other hand, Young et al. (2002) observed an association between the *A1* allele and

PTSD, but this association was only observed in patients with PTSD who were classified as 'harmful drinkers' (i.e. consuming >60 g alcohol/day). In a more recent study, Bailey et al. (2010) found no association between *DRD2 Taq1A* and PTSD.

Brain-derived neurotrophic factor (BDNF) has also been implicated in the development of PTSD. BDNF is widely expressed throughout the mammalian brain, and is involved in neurodevelopment, neuronal survival, neuronal morphology and differentiation, synaptic plasticity and protection against stress-induced neuronal damage (Bergstrom et al., 2008; Duman and Monteggia, 2006; Hoglinger et al., 1998; Huang and Reichardt, 2001; Lu, 2003; Poo, 2001). There is also evidence from animal studies that psychological or physical stress, or both, may inhibit hippocampal BDNF expression (Duman et al., 1997, 2000; Kozlovsky et al., 2007; Smith et al., 1995). In addition, serum levels of BDNF have been found to be decreased in individuals with PTSD compared to healthy controls (Dell'osso et al., 2009). Karege et al. (2002) previously demonstrated a correlation between levels of BDNF in the serum and central nervous system (CNS). A SNP in the BDNF gene (*Val66Met*; rs6265) has been found to affect dendritic trafficking, synaptic localisation and activity-dependent secretion of BDNF (Chen et al., 2006; Egan et al., 2003). Individuals who carry the *Met66* allele have been found to exhibit poorer episodic memory and abnormal hippocampal activation (Egan et al., 2003), as well as reduced hippocampal volumes (Bueller et al., 2006; Hajek et al., 2012; Montag et al., 2009; Pezawas et al., 2004), often observed in patients with PTSD (Bremner et al., 1997, 2008; Sapolsky, 2000). The *BDNF Met66* allele has also been found to be associated with impaired fear extinction in both a mouse model and human cohort (Soliman et al., 2010; Yu et al., 2009). Although previous studies found no association between *BDNF Val66Met* and PTSD (Lee et al., 2006; Valente et al., 2011; Zhang et al., 2006), sample sizes were relatively small in these studies, and none of them accounted for gene–gene interactions, warranting further investigation.

The sample in the present study was drawn from four communities of low socioeconomic status in the Western Cape, namely Ravensmead, Uitsig, Adriaanse and Elsies River. A large proportion of the residents from these areas are unemployed, and those that are employed receive annual incomes of between R19,201 (US equivalent $2300) and R76,800 (US equivalent $9183). In addition to being very poor, these areas have a very high tuberculosis (TB) incidence (Kritzinger et al., 2009). Crime and violence are also common in this area. Ravensmead has been named as a "high-risk area" for gang-related serious violent crime by the Western Cape Organised Crime Unit of the South African Police Service (SAPS) (Kagee and Frank, 2005). Dinan et al. (2004) found that South African women from a similarly low socioeconomic area characterised by high levels of both domestic and community violence reported a median of three traumatic incidences in the previous 12-month period. Moreover, a PTSD rate of 25.8%, as assessed by a self-report measure of PTSD, has previously been documented in a study conducted in an urban community sample of adult victims of violent crime in South Africa (Peltzer, 2000). The aforementioned rate of PTSD is considerably higher than that found in the recently conducted South African Stress and Health (SASH)

study. The SASH study, which was conducted among a national representative sample of 4351 adults, reported an estimated 12-month prevalence for PTSD of 0.6%, with an estimated lifetime prevalence rate for PTSD of 2.3% (Herman et al., 2009). Thus, the study population represents a population at high risk for experiencing trauma and for developing subsequent stress-related disorders.

The aim of the current study was to determine whether genetic factors play a role in the development and severity of PTSD symptoms in a population that is at high risk for experiencing trauma. To this end, we investigated the association between specific alleles or genotypes of (1) *5-HTTLPR*, (2) *DRD2 Taq1A* and (3) *BDNF Val66Met* and trauma symptom severity in a vulnerable population. We also investigated whether epistatic interactions between the genetic variants influenced trauma symptom severity.

## 2. Methods

### *2.1 Participants*

One hundred and fifty participants were drawn from an ongoing study investigating the effect of psychological stress on the development of TB in close contacts of TB patients (unpublished data). All participants were trauma-exposed, close household contacts of patients with active TB disease, diagnosed through four community health clinics in the Cape Town area. The participants were all non-TB cases, and all identified themselves as Coloured (mixed race). The protocol was approved by the Health Research Ethics Committee at Stellenbosch University, and all subjects provided written, informed consent after being presented with a complete description of the study.

### *2.2 Measurements: demographic characteristics*

A researcher-administered questionnaire was used to collect information on participants' age, marital status, level of education, annual household income and employment status.

### *2.3 Clinical measures*

Psychiatric morbidity, including PTSD, was assessed using the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998), a clinician-administered, structured diagnostic interview for major psychiatric disorders based on the DSM-IV diagnostic criteria.

The 18-item Traumatic Life Events Checklist (LEC) was used to identify exposure to potentially traumatic events that participants may have directly experienced, witnessed or learned about. Once the most traumatic event was specified by the participant, the Davidson Trauma Scale (DTS) (Davidson et al., 1997) was administered to those participants who endorsed trauma exposure so as to determine the severity of posttraumatic stress symptoms. The DTS is a 17-item self-report measure that assesses both the frequency and intensity of PTSD symptoms among individuals with a history of trauma exposure. Items are measured on a 5-point Likert-type scale, ranging from 0 ('not at all') to 4 ('every day/extreme'), with higher scores

depicting more severe PTSD symptoms. A total score of greater than, or equal to, 40 has been found to most accurately predict clinical diagnosis of PTSD (Davidson et al., 1997).

Perceived social support was assessed using the Multidimensional Scale of Perceived Social Support (MSPSS) (Zimet et al., 1988), a 12-item, self-report measure that assesses perceptions of social support from three sources, namely family, friends and significant others. The items are measured on a 7-point scale, ranging from 1 ('very strongly disagree') to 7 (very strongly agree), with higher scores indicating a greater degree of social support. Because social support has been found to represent a risk/resiliency factor in PTSD (Brewin et al., 2000; Ozer et al., 2003), it was necessary to assess and correct for this variable.

Resilience was measured using the Connor–Davidson Resilience Scale (CD-RISC) (Connor and Davidson, 2003). This is a 25-item self-report scale that measures stress coping ability. Items are measured on a 5-point scale from 0 ('not true at all') to 4 ('true nearly all of the time'). A total score between 0 and 100 is obtained, with higher scores indicating increased resilience.

### *2.4. Genotyping*

DNA was extracted from whole blood, using the Nucleon BACC3 Illustra extraction kit (GE Healthcare). The loci containing each of the polymorphisms were amplified individually, using previously published primers (Baune et al., 2008; Grandy et al., 1993; Sen et al., 2003). Thermal cycling was performed in a GeneAmp® PCR system 9700 (Perkin Elmer Biosystems, Foster City, CA, USA) for 30 cycles for all polymorphisms. The annealing temperatures and length of the PCR-amplified product for each set of oligonucleotide primers are presented in Table 1.

The long allele (*L*) of *5-HTTLPR* is characterised by a size of 528 bp, while the short allele (S) is characterised by a size of 484 bp (Table 1). The *5-HTTLPR* amplicons were electrophoresed on 2% ethidium bromide-stained agarose gels, and sized accordingly. Allele-specific restriction enzyme analysis (ASREA) was performed to characterise the genotypes arising from the *DRD2 Taq1A* and *BDNF Val66Met* polymorphisms as follows.

For *DRD2 Taq1A*, the 310 bp amplicon was digested overnight at 65 °C with 10 U of *Taq*1 (New England Biolabs, Beverly, MA, USA). The restriction enzyme recognizes a site in the *A2* allele, resulting in two DNA fragments of 130 bp and 180 bp, whereas the *A1* allele remains uncut as a 310 bp fragment. *DRD2 Taq1A* alleles were visualised on 2% agarose gels, stained with ethidium bromide. For *BDNF Val66Met*, the 274 bp amplimer was digested with 5U *Nla*III (New England Biolabs, Beverly, MA, USA) overnight at 37 °C. Two *Nla*III restriction enzyme sites are present in the amplified genomic fragment. The first site is cut constitutively and produces fragment sizes of 57 bp and 217 bp, whereas at the polymorphic site, *Nla*III cuts the *A* (*Met66*) allele, generating fragments of 140 bp, 77 bp and 57 bp in

size, while the *G* (*Val66*) allele remains uncut. *BDNF Val66Met* alleles were visualised on 3% agarose gels, stained with ethidium bromide.

### *2.5. Statistical analysis*

Data was summarised as median (interquartile range [IQR]) for all quantitative variables, as most had very skewed, and therefore non-normal, distributions. Categorical traits, including genotypes, were summarised as counts and percentages for the study group as a whole, and for males and females separately. The appropriate unadjusted tests provided the p-values for sex differences in the variables.

The DTS scores did not possess a standard distribution, as a preponderance of zeros was observed (53 [35%]). To obtain valid results, a zero-inflated analysis model was required (Zeileis et al., 2008). This analysis represents a mixture of two models: one model fits a count distribution to the data, the second (binomial) makes a percentage of those values zero. We used a zero-inflated model with a negative binomial family for the (non-zero) counts.

Genetic association and interactions were tested by including single genotype and genotype-by-genotype interaction terms, respectively, as categorical fixed effects in the models. Interaction terms that were not significant were removed from the models. When inspection of genotype effects indicated that the heterozygote and the minor homozygote had similar effects, a dominant coding of the genotype was tested.

The analysis was adjusted for known confounders, namely age, sex, the presence or absence of major depression, alcohol dependence, resilience, social support, and levels of education and employment. These confounders were adjusted for by including them in the statistical models as fixed effects. The analysis yields two sets of results, corresponding to the mixture of two models, for each factor (confounders and polymorphisms investigated): first, whether it affects the zero or non-zero status of DTS, and second, what the count is, if it is not zero. The significance of effects (p-values) and effect sizes (% differences) are part of the results of the confounder-adjusted zero-inflated models used in the analyses.

Statistical analyses were done with functions from R (www.r-project.org), and R packages *genetics* (genotype and allelic counts and frequencies; Hardy-Weinberg equilibrium testing) and *pscl* (zero-inflated modelling).

**Table 1**
Description of the candidate polymorphisms used in the genetic association analyses.

| Gene | DNA variant | Annealing temperature (°C) | Amplicon size (bp) | Reference |
|---|---|---|---|---|
| *5-HTT* | *5-HTTLPR* | 60 | *L*-allele: 528 bp<br>*S*-allele: 484 bp | Baune et al. (2008) |
| *DRD2* | *Taq1A* (rs1800497) | 60 | 310 bp | Grandy et al. (1993) |
| *BDNF* | *Val66Met* (rs6265) | 63 | 274 bp | Sen et al. (2003) |

Abbreviations: bp, base pairs; *5-HTT*, serotonin transporter; *5-HTTLPR*, serotonin transporter gene-linked polymorphic region; *DRD2*, dopamine receptor 2; *BDNF*, brain-derived neurotrophic factor.

## 3. Results

### *3.1. Demographic and clinical measures*

Table 2 summarises the demographic and clinical variables measured in the total study group, and stratified by sex. Thirty-one percent (47/150) of the study group was male. The median age of the group was 30.5 years (IQR: 23–42 years), with no significant differences observed between male and female participants (p= 0.440). Fifty-nine participants (39.3%) possessed an education level of Grade 8 (equivalent to ten years of schooling) or less, and no significant differences were observed with regard to level of education between male and female participants (p= 0.858). Only 32.7% of the participants were employed, with significantly more males being employed (pb 0.001). More than half of the participants reported an annual income of less than R10,000 (US equivalent $1205), indicating that the majority were living in poverty.

**Table 2**
Demographic and clinical variables of the study group, stratified by sex.

| Variable | Group | Male | Female | p-Value |
|---|---|---|---|---|
| Age in years, median (IQR) | 30.5 (23–42) | 30 (23–44) | 33 (24–33) | 0.440 |
| Education (<Grade 8) | 59 (39.3) | 18 (38.3) | 41 (39.8) | 0.858 |
| Employed (yes/no) | 49 (32.7) | 28 (60) | 21 (20) | <0.001 |
| CD-RISC score, median (IQR) | 73 (58–84) | 71 (60–86) | 73 (58–83) | 0.711 |
| MSPSS, median (IQR) | 64 (51–73) | 63 (56–72) | 64 (48–73) | 0.984 |
| MDD diagnosis | 37 (25) | 10 (21) | 27 (26) | 0.548 |
| Alcohol dependence | 24 (16) | 12 (25.5) | 12 (11.7) | 0.030 |
| DTS (non-zero scores), median (IQR) | 35 (17–58) | 25.8 (11–61) | 39 (20–58) | 0.046 |

Unless otherwise indicated, all values are provided in counts (percentages).
Abbreviations: IQR, interquartile range; CD-RISC, Connor–Davidson Resilience Scale; MSPSS, Multidimensional Scale of Percieved Social Support; MDD, major depressive disorder; DTS, Davidson Trauma Scale.

**Table 3**
Genotype counts and percentages for *5-HTTLPR*, *DRD2 Taq1A* and *BDNF Val66Met* the total study group, and stratified by sex.

| Gene variant | Genotype | Total n (%) | Male n (%) | Female n (%) |
|---|---|---|---|---|
| *5-HTTLPR* | *L/L* | 68 (47.2) | 21 (47.7) | 47 (47.0) |
| | *L/S* | 55 (38.2) | 17 (38.6) | 38 (38.0) |
| | *S/S* | 21 (14.6) | 6 (13.6) | 15 (15.0) |
| *BDNF Val66Met*[a] | *Val/Val* | 115 (76.7) | 35 (74.5) | 80 (77.7) |
| | *Val/Met* | 33 (22.0) | 11 (23.4) | 22 (21.4) |
| | *Met/Met* | 2 (1.3) | 1 (2.1) | 1(1.0) |
| *DRD2 Taq1A*[b] | *A2/A2* | 65 (43.3) | 20 (42.6) | 45 (43.7) |
| | *A1/A2* | 70 (46.7) | 22 (46.8) | 48 (46.6) |
| | *A1/A1* | 15 (10.0) | 5 (10.6) | 10 (9.7) |

Abbreviations: *5-HTTLPR*, serotonin transporter linked polymorphic region; *BDNF*, brain-derived neurotrophic factor; *DRD2*, dopamine receptor 2.
[a] *Val* allele corresponds to the *G*-allele, *Met* allele corresponds to *A*-allele.
[b] *A1* allele corresponds to the *C*-allele, *A2* allele corresponds to *T*-allele.

According to the M.I.N.I, seventeen participants met the criteria for current PTSD diagnosis (11.3%). The median scores for CD-RISC and MSPSS were 73 (IQR: 58–84) and 64 (IQR: 51–73), respectively, with no sex differences observed for either scores (p= 0.711 [CD-RISC] and p= 0.984 [MSPSS]) (Table 2). Thirty-seven (25%) of the study participants were diagnosed with major depressive disorder (MDD) (10 [21%] male; 27 [26%] female; p= 0.548). Twenty-four individuals (16%) met criteria for alcohol dependence, and of those, 12 (50%) were male. Males were significantly more likely to exhibit alcohol dependence compared to females (p= 0.030).

According to the LEC, 99% of the participants (149/150) had experienced, witnessed or learned about a traumatic event (s). A DTS score of zero indicated that either the participant had not experienced a traumatic event, or that the traumatic event that was experienced did not result in the development of PTSD symptoms. Fifty-three participants (35%) reported zero DTS scores, while 97 participants (65%) reported non-zero DTS scores. The median of the DTS non-zero scores was 35 (IQR: 17–58). In a joint analysis of association of the potential confounders with DTS, none were found to be significantly associated with the zero/non-zero status of an individual's DTS score. However, three of the potential confounders, namely sex, MDD status and level of social support, were found to contribute significantly to non-zero DTS score. To this end, males were found to have significantly lower DTS scores than females (effect= 29%; 95% CI: 1–49%; p= 0.046), while being diagnosed with MDD increased DTS score significantly (effect= 78%; 95% CI: 25–153%; p= 0.001). In addition, an increased level of social support was found to reduce the DTS score significantly (effect of each additional score on MSPSS scale= 2%; 95% CI: 1–3%; p b 0.001).

### *3.2 Genotype data and allele frequencies*

All variants were in Hardy–Weinberg equilibrium (*5-HTTLPR* p= 0.093; *DRD2 Taq1A* p= 0.587; *BDNF Val66Met* p= 1.000). Genotype counts and percentages for each polymorphism, in the total study group and stratified by sex, are provided in Table 3. No significant differences in genotype and allele frequencies were noted between males and females (*5-HTTLPR* p= 0.977 [genotype] and p= 0.871 [allele]; *DRD2 Taq1A* p= 0.981

[genotype] and p= 0.857 [allele]; *BDNF Val66Met* p= 0.817 [genotype] and p= 0.594 [allele]).

### *3.3 Association of genetic variants with severity of trauma symptoms*

Although the *5-HTTLPR* variant was not associated with the non-zero DTS score (p= 0.444), participants who possessed at least one *5-HTTLPR S*-allele (dominant model) were significantly more likely to be scored as 'zero' on the DTS scale (odds ratio [OR]= 2.77; 95% CI: 1.28–5.97; p= 0.012).

An epistatic interaction between *BDNF Val66Met* and *DRD2 Taq1A* polymorphisms was found to impact non-zero DTS score (p= 0.006). The nature of this significant interaction is illustrated by box plots of the observed non-zero DTS scores in Fig. 1. It was found that, on the background of the *BDNF Val66* homozygote, DTS score increased significantly with the addition of each *DRD2 Taq1A A1* allele. However, upon the *BDNF Met66* background, the DTS score was found to increase significantly on addition of each *A2* allele. No statistically significant differences were observed with regard to zero or non-zero status of DTS scores between the genotypes or alleles comprising the individual *DRD2 Taq1A* or *BDNF Val66Met* polymorphisms.

## 4. **Discussion**

The study group was found to exhibit a current PTSD prevalence of 11.3%, which is considerably higher than the 12-month and lifetime prevalence rates observed in the recently conducted SASH study (Herman et al., 2009), indicating that the population is indeed at increased risk for developing the disorder.

We found that individuals possessing the *5-HTTLPR LL* genotype were significantly more likely to have a non-zero DTS score compared to those who possessed at least one *S* allele. In the present study, a score of 'zero' on the DTS indicated either that the individual had not experienced a traumatic event that had significantly impacted their life, or that, although the individual had experienced a traumatic event (s), it had not resulted in any PTSD symptoms. Only one participant in the present study indicated that they had not experienced any traumatic event. This finding therefore suggests that those individuals who possessed at least one *S* allele were more resilient to the effects of trauma compared to those carrying the *LL* genotype. Although the *S* allele has been found to be associated with reduced resilience in a recent study (Stein et al., 2009), the *L*-allele has also been found to be associated with reduced resilience in subjects with insecure attachment (Olsson et al., 2005) and those who had been exposed to early adversity (Carli et al., 2011; Laucht et al., 2009). Given the inconsistent results in publications investigating resilience and *5-HTTLPR*, the current study needs to be replicated in a larger sample in order to validate the results presented here and to better understand the relationship between the two variables.

To our knowledge, this is the first published study to suggest a role for epistatic interaction between the *DRD2 Taq1A* and *BDNF Val66Met* polymorphisms in the severity of PTSD symptoms. We found that, on a background of the *BDNF Val66Val* genotype, DTS score increased significantly with the addition of each *DRD2 Taq1A A1* allele.

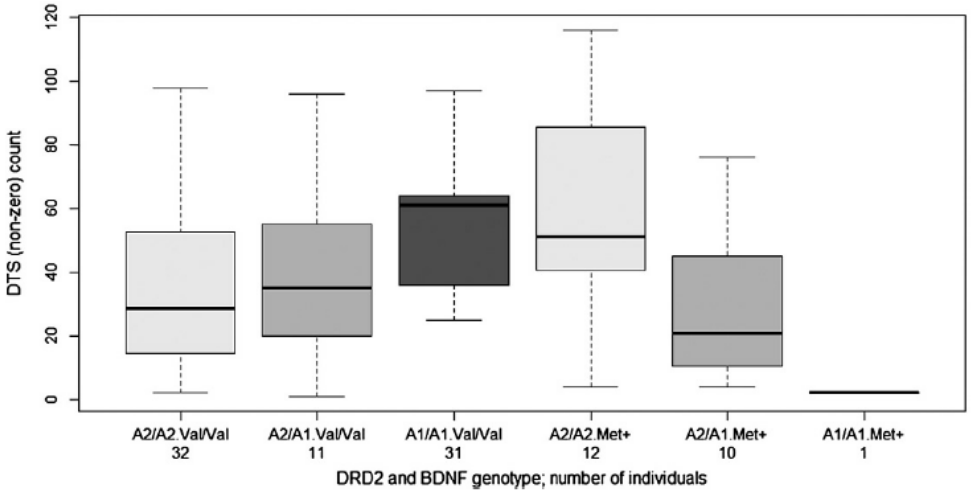


**Fig. 1.** Boxplots summarising the observed distribution of non-zero DTS (n = 97), stratified by combined genotype. The boxes indicate the middle 50% of the values while the whiskers extend to the minimum and maximum, respectively. Abbreviations: DTS, Davidson Trauma Scale; DRD2, dopamine receptor 2; BDNF, brain-derived neurotrophic factor. *Val* allele corresponds to the *G*-allele, *Met* allele corresponds to *A*-allele. *A1* allele corresponds to the *C*-allele, *A2* allele corresponds to *T*-allele.

Interestingly, however, on the background of the *BDNF Val66Met/Met66Met* (*Met66+*) genotype, DTS score increased significantly with each additional *DRD2 Taq1A A2* allele. Although neither of the genetic variants was found to contribute to PTSD symptom severity individually in the present study, prior research has suggested the involvement of the *A1* allele in the aetiology of PTSD (Comings et al., 1996; Young et al., 2002), although Young et al. (2002) only observed this association in individuals who exhibited harmful drinking. Given the role of BDNF in a number of crucial processes in the CNS, mounting evidence suggests that BDNF may also play a role in the development of PTSD. However, to date, only three published studies have investigated the role of *BDNF Val66Met* in the aetiology of the disorder, all yielding negative results (Lee et al., 2006; Valente et al., 2011; Zhang et al., 2006).

It is perhaps not surprising that the *BDNF* and *DRD2* polymorphisms were found to interact to influence DTS score in the present study, since BDNF has previously been found to be involved in the modulation, maintenance and normal functioning of mesolimbic dopaminergic neurotransmission (Berton et al., 2006; Bustos et al., 2004; Goggi et al., 2003; Hunnerkopf et al., 2007) and in the development of dopaminergic neurons (Baquet et al., 2005; Trzaska et al., 2009). In Fig. 1, it is noteworthy that the influence of epistatic interaction between *BDNF Val66Met* and *DRD2 Taq1A* polymorphisms on DTS score approximates an inverted U-shaped curve. This observation may be best explained by previous research indicating that dopamine functions within a range of optimal concentrations to affect certain brain functions; for example, both too much dopamine and too little dopamine have been found to impair cognitive functionality (Cools and D'Esposito, 2011; Goldman-Rakic et al., 2000). This U-

shaped function of dopamine has previously been thought to be due to alterations in dopamine D1 receptor activity (Cools and D'Esposito, 2011; Seamans and Yang, 2004), but it has recently been reported that DRD2 activation also affects dopamine concentrations in a non-linear fashion, and that an optimal range of DRD2 activity exists to facilitate adequate brain functioning (Gjedde et al., 2010; Monte-Silva et al., 2009). Notably, *DRD2 Taq1A* has also been implicated in affecting the amount of available dopamine in various regions of the brain during feedback-guided learning (Cohen et al., 2007), implicating this variant in the U-shaped effect that dopamine has on certain brain functionality. Although the precise relationship between *BDNF Val66Met* and *DRD2 Taq1A* variants is yet to be elucidated, it has been postulated that a reduction in *BDNF* expression results in reduced dopaminergic activity in the mesolimbic pathways leading to the anterior cingulate cortex (ACC) (Koven and Carr, 2012).

Given the aforementioned information and from the results obtained in the present study, it is tempting to speculate that the *BDNF Val66Met* genotype drives the U-shaped functionality of dopamine in the context of PTSD symptom severity. In other words, an increased BDNF activity-dependent secretion (due to the genotype *Val66Val* (Egan et al., 2003)) may, together with the reduced *DRD2* expression caused by the *DRD2 Taq1A A1/A1* genotype (Jonsson et al., 1999; Noble et al., 1991; Pohjalainen et al., 1998; Ritchie and Noble, 2003; Thompson et al., 1997), cause a significant increase in dopaminergic concentration. On the other hand, the reduced BDNF activity-dependent secretion (due to *Met66*-containing genotypes (Egan et al., 2003)) and the increased *DRD2* expression (due to the *DRD2 Taq1A A2/A2* genotype (Jonsson et al., 1999; Noble et al., 1991; Pohjalainen et al., 1998; Ritchie and Noble, 2003; Thompson et al., 1997)) may, together, significantly reduce dopaminergic concentration. The epistatic interactions between the two genes may therefore render the dopaminergic levels sub-optimal for cognitive functions that impact PTSD symptom severity.

Epistasis between the *BDNF Val66Met* and *DRD2 Taq1A* variants has previously been observed in the context of personality dimensions of harm avoidance and novelty seeking (Montag et al., 2010a) and alexithymia (Walter et al., 2011), and volume in the ACC (Montag et al., 2010b). Montag et al. (2010a) found that healthy individuals who possessed at least one *Met66* and one *A1* allele (*Met66+/A1+* combination) exhibited significantly increased scores on the Harm Avoidance scale and significantly reduced scores on the Novelty Seeking scale. In a separate publication by the same group, the same combination of *DRD2/BDNF* genotypes was found to be associated with significantly reduced grey matter volume in the ACC (Montag et al., 2010b). Individuals with the *Met66+/A1+* genotype combination have also been found to be more likely to exhibit increased levels of alexithymia (Walter et al., 2011), a personality construct that involves difficulties in identifying emotional states, and an inability to verbalise one's own emotions (Larsen et al., 2003). Interestingly, both increased levels of alexithymia (Declercq et al., 2010) and reduced ACC volume (Kasai et al., 2008; Kitayama et al., 2006; Thomaes et al., 2010; Woodward et al., 2006) have been observed in PTSD patients.

Therefore, the interaction between *DRD2* and *BDNF* may increase the risk of developing PTSD by reducing ACC volume and increasing symptoms of alexithymia observed in PTSD.

### *4.1. Study limitations*

A number of limitations in the present study deserve mention. The study group size of 150 participants is relatively small; consequently, the present results require validation in studies comprising larger sample sizes, which will impart greater power. In addition, population stratification was not controlled for, which may have confounded results. The South African Coloured population has a unique mixed ancestry dating back almost 300 years and comprises a number of different ethnicities, including European Caucasoid, Asian, Khoi, San and Black African (Nurse et al., 1985). Since the admixture occurred several generations ago, the population has been suggested to be relatively genetically homogenous. In a recent study in which 25 unlinked markers were genotyped in a South African Coloured population comprising 351 TB cases and 360 controls, no significant population stratification was observed (Barreiro et al., 2006). However, in a subsequent study, de Wit et al. (2010) observed substantial genetic heterogeneity within the South African Coloured population. Future studies should thus include measures to correct for population stratification within the South African Coloured population.

It must also be noted that multiple testing was not corrected for, primarily due to the exploratory nature of the present study. In addition, the most appropriate means of correcting for multiple testing remains contentious and applying Bonferroni's correction may be too conservative in cases where there is prior evidence that such associations may be present.

In light of the exploratory nature of the study, it was decided to limit the genetic analysis of the serotonin transporter to one polymorphism, namely *5-HTTLPR*. However, the authors are mindful of the presence of additional polymorphisms within the gene (such as rs25331 (Hu et al., 2006) and rs25532 (Wendland et al., 2008)) that may modulate the functionality of *5-HTTLPR*. Investigating these polymorphisms will form an important aspect of future validation studies.

It is possible that other gene variants may mediate the interaction between the *BDNF Val66Met* and *DRD2 Taq1A* polymorphisms. For example, the catechol-O-methyltransferase (*COMT*) *Val158Met* variant has been found to alter the functionality of COMT, which is an important regulator of dopaminergic neurotransmission, and has also been found to be involved in reduction in ACC volume in patients with PTSD (Schulz-Heik et al., 2011). Future studies should thus include additional variants, such as *COMT Val158Met*.

Finally, the aetiology of psychiatric disorders, and particularly PTSD, involves a large environmental component, thought to interact with the genetic component. It is therefore important that future studies take environmental factors into account.

## 5. Conclusions

The preliminary results yielded in the present study implicate the interaction between genes encoding BDNF and DRD2 in the severity of PTSD symptoms. As mentioned, the present results require validation in a larger study group, while accounting for environmental factors that may contribute to the development of PTSD, and possible population stratification. If the present results are validated, further work will be required to determine the mechanism of action of the interaction between BDNF and DRD2. These results could have important implications for novel therapeutic regimes with which to treat this debilitating psychiatric disorder.


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## Contributors

Sian Hemmings wrote the first draft of themanuscript; LindiMartin recruited and assessed the participants; Lisa Aitken assessed participants; Lize van der Merwe performed the statistical analyses and wrote parts of the manuscript; Marisa Klopper and Erika de Wit performed genetic analyses; Eileen Hoal provided access to blood samples; Gillian Black,GerhardWalzl and Soraya Seedat designed the study. All authors contributed to and have approved the final manuscript.


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